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The Characterization of the Binding of Opacity-Associated Proteins to Human Host Cell Receptors

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Opacity-associated (Opa) proteins are eight-stranded β-barreled monomeric outer membrane proteins found in the bacterial pathogens *Neisseria meningitides* and *N. gonorrhoeae*. *In vivo*, these proteins interact with specific human host cell receptors to induce phagocytosis of the bacterium, allowing *Neisseria* to breach the plasma membrane and gain entry to targeted human cells. There are at least 26 characterized Opa proteins, nearly identical in sequence except for three extracellular loops. Host-receptor specificity is determined by the variable extracellular loops; however, the specific molecular determinants of the interaction have not been identified. Opa proteins are classified into two families based on their host receptor selectivity, the larger class, Opa_{CEA}, bind to carcinoembryonic antigen-like cellular adhesion molecules (CEACAMs) and the smaller class, Opa_{HS}, bind to heparansulfate proteoglycan receptors (HSPGs) or to integrin receptors via an HSPG-mediated interaction with fibronectin or vitronectin. Though the Opa-host receptor interaction is directly related to the invasion efficiency of *Neisseria*, the thermodynamics (e. g. affinities) of Opa proteins interactions with host receptors is not known. We present a study investigating the binding of two Opa protein variants, OpaI (an Opa_{CEA}) and OpaA (an Opa_{HS}) with their cognate host receptors. A centrifugal ''pull-down'' assay is used with fluorescence spectroscopy to determine the specificity and affinity of the Opa - receptor interactions. Although attempts have been made to study Opa specificity *in vivo*, this study demonstrates Opa protein selective binding to the respective receptors *in vitro*, thereby facilitating investigations of protein determinants relating both to binding specificity and affinity.

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Tyrosine Replacement Diminishes Association of PSGL-1 with P- and L-Selectins on the Cell Membrane

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Binding of selectins to P-selectin glycoprotein ligand-1 (PSGL-1) mediates tethering and rolling of leukocytes on endothelium during inflammation. While mutations of three tyrosines at the PSGL-1 N-terminus has been shown to increase the reverse rates and their sensitivity to force of bonds with P- and L-selectins [(1)], the roles of the mutations on the binding affinities and forward rates had not been studied well yet. Here we quantified these effects using an adhesion frequency assay for measuring two-dimensional (2D) affinity and kinetic rates at zero force [(2)]. Comparing to wild-type PSGL-1, binding affinities for P- and L-selectin was 7-164-fold lower for PSGL-1 mutants with two of three tyrosines substituted by phenylalanines and 89-284-fold lower for PSGL-1 mutant with all three tyrosines replaced. These differences were attributed to enhancements in forward rates without major changes in reverse rates, suggesting that these tyrosines regulate the accessibility of PSGL-1 to selectins and that at least one of the three tyrosines is required for selectin-PSGL-1 binding. Our results provided an insight into understanding the receptor-ligand binding at a single residue level.

1. V. Ramachandran *et al.*, *Proc.Natl.Acad.Sci.USA* (1999).
2. J. Huang *et al.*, *Nature* (2010).

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The Hydrophobic Proteins of Pulmonary Surfactant Reduce Bilayer Elasticity

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Pulmonary surfactant is a complex mixture of phospholipids and proteins that lowers surface tension at the alveolar air-water interface. The hydrophobic proteins SP-B and SP-C drive adsorption of surfactant vesicles to form the interfacial film. To investigate the structural basis underlying the function of the proteins, we used small angle x-ray scattering (SAXS) and 31P NMR to correlate a reduction in membrane elasticity and the formation of highly curved structures, with elevated rates of adsorption. Multilamellar vesicles were prepared using dioleoyl phosphatidylcholine (DOPC) with varying concentrations of the two proteins in their physiological ratios. SAXS patterns exhibited peak-broadening as well as linear increases in the interlamellar d-spacing with greater protein concentrations. In the absence of protein, global analysis of the patterns yielded a relatively low Caillé parameter ($\eta \sim 0.1$), indicating minor bilayer undulations. Incorporating protein into the vesicles induced large increases in the Caillé parameter from 0-4% (w:w), suggesting significant decreases in membrane elasticity as a function of protein concentration. 31P NMR spectra for the same samples showed the presence of an isotropic phase at concentrations as low as 0.25%, which grew in a dose-dependent manner with increasing concentrations of protein. Measured adsorption rates also exhibited dose-dependence in parallel with the SAXS and NMR data. The induced decrease in membrane bending rigidity, indicated by an increased Caillé parameter, and the formation of an accompanying isotropic phase, shown by the NMR spectra, suggests that these structural developments are directly related to the function of the surfactant proteins.

SAXS data was collected at the Stanford Synchrotron Radiation Lightsource.

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Probing Skeletal Dysplasias Caused by Mutations of FGFR3 Using Qi-FRET

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Fibroblast growth factor receptor 3 (FGFR3) is a member of the receptor tyrosine kinase (RTK) family. FGFR3 is a single pass transmembrane protein and is involved in cell signaling, regulation, and proliferation. Signals are mediated via the lateral dimerization of the transmembrane spanning proteins and their interactions with extracellular ligands. It is important for these signaling pathways to be tightly regulated. Several mutations in FGFR3 are known to result in unregulated activation and/or disruption of proper cellular functions leading to pathogenesis. For example, single point mutations in FGFR3 are implicated in diseases such as achondroplasia, a form of dwarfism, and thanatophoric dysplasia, which is a lethal form of skeletal dysplasia. We are currently probing the interaction of mutant versions of FGFR3 to determine how these interactions change relative to that of the wild type FGFR3. These interactions are quantified via QI-FRET. In this approach, CHO cell-derived plasma membrane vesicles are prepared to mimic the native environment for the expressed FGFR3 constructs, which are linked to fluorescent proteins for direct visualization. Two important attributes of this approach over conventional whole-cell imaging are the low background noise and the strong intensity of the obtained vesicles. This results in the ability to fit the intensity profile with a Gaussian distribution and facilitate automated processing of images such that large numbers of vesicles with varying protein concentrations can be analyzed. From which, changes in interactions between wild type and mutant FGFR3 can be quantitatively determined.

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Kinetics of the Cytochrome C Oxidase from *R. Sphaeroidis* Under Turn-Over Conditions by Time-Resolved Surface-Enhanced Infrared Absorption Spectroscopy (SEIRas)

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Cytochrome c Oxidase (CcO) is investigated under conditions of electronic wiring to a gold electrode modified with Au nanoparticles, designed for use with SEIRA spectroscopy in the ATR mode. In the presence of oxygen cyclic voltammetry had indicated that the enzyme is catalytically active when it is immobilized in the orientation with the first electron acceptor Cu_A directed toward the electrode. After immobilization CcO was protected by a lipid bilayer generated by in-situ dialysis. The full catalytic activity was obtained only after the enzyme had been subjected to a succession of several turn-overs. Thereafter SEIRA spectra measured under static conditions had indicated that the enzyme was in an ¯activated¯ state considered equivalent to the pulsed state found in biochemical assays.

Time-resolved (tr) SEIRA spectra recorded in the step-scan mode of the CcO were initiated by periodic potential pulses changing between an oxidizing and reducing potential thus varying the redox state of the enzyme many times. This allowed us to measure the kinetics of electron transfer at different frequencies. Moreover, amino acids correlated with electron transfer (ET) to the redox centers Cu_A, heme a, heme a_3 and Cu_B were identified. Particularly useful in this context proved 2D IR auto-correlation maps, which also allowed us to identify the sequence of conformational changes regarding different amino acids involved in ET and proton transfer as well as the reorientation of the transition dipole moments of various groups relative to each other.

Christoph Nowak, M. Gabriella Santonicola, Denise Schach, Jiapeng Zhu, Robert B. Gennis, Dieter Baurecht, Thamara Laredo, Jacek Lipkowsky, Dieter Walz, Wolfgang Knoll, Renate L. C. Naumann, Soft Matter, 2010, DOI:10.1039/C0SM00160K.